WHAT IS CLAIMED IS:

1. A nucleic acid ligation assay comprising:

contacting a sample suspected of containing one or more target nucleic acid sequences with one or more subsets of free probes and one or more subsets of spectrally-addressable bound probes;

allowing the one or more subsets of free probes and one or more subsets of spectrally-addressable bound probes to hybridize to the one or more target nucleic acid sequences, if present;

ligating the hybridized free probes with the hybridized spectrally-addressable bound probe, wherein a free probe hybridized to a target nucleic acid sequence is ligated with a spectrally-addressable bound probe hybridized to the same target nucleic acid sequence, to provide spectrally-addressable ligated products; and at least one of

detecting the presence of the spectrally addressable ligated products or analyzing the nucleic acid sequence of the spectrally-addressable ligated products.

2. An assay according to claim 1, wherein the sample is suspected of containing two or more target nucleic acid sequences, the sample is contacted with two or more subsets of spectrally-addressable bound probes, wherein one subset of bound probes is distinguishable from other subsets of bound probes at least based on the nucleotide sequence at the free end of the probes.

3. An assay according to claim 1, wherein:

the sample is suspected of containing one or more first and one or more second target nucleic acid sequences, the one or more first target nucleic acid sequences have at least a first portion and a second portion and the one or more second target nucleic acid sequences have at least a first portion and a second portion, and wherein the first portion of the one or more first target

nucleic acid sequences is distinguishable from the first portion of the one or more second target nucleic acid sequences but the second portion of the one or more first target nucleic acid sequences is substantially identical to the second portion of the one or more second target nucleic acid sequences; and

the sample is contacted with two subsets of spectrally-addressable bound probes and one subset of free probes, wherein the first subset of spectrally-addressable bound probes is specific for the first portion of the one or more first target nucleic acid sequences and the second set of spectrally-addressable bound probes is specific for the first portion of the one or more second target nucleic acid sequences and the free probes have substantially identical nucleotide sequences specific for the second portion of the one or more first and second target nucleic acid sequences.

4. An assay according to claim 1, wherein the sample is suspected of containing two or more target nucleic acid sequences, the sample is contacted with two or more subsets of free probes, wherein one subset of free probes is distinguishable from other subsets of free probes at least based on the nucleotide sequence at the free end of the probes.

5. An assay according to claim 1, wherein:

the sample is suspected of containing one or more first and one or more second target nucleic acid sequences, the one or more first target nucleic acid sequences have at least a first portion and a second portion and the one or more second target nucleic acid sequences have at least a first portion and a second portion, and wherein the first portion of the one or more first target nucleic acid sequences is distinguishable from the first portion of the one or more second target nucleic acid sequences but the second portion of the one or more first target nucleic acid

sequences is substantially identical to the second portion of the one or more second target nucleic acid sequences; and

the sample is contacted with two subsets of free probes and one subset of spectrally-addressable bound probes, wherein the first subset of free probes is specific for the first portion of the one or more first target nucleic acid sequences and the second set of spectrally-addressable bound probes is specific for the first portion of the one or more second target nucleic acid sequences and the one subset of spectrally-addressable bound probes have substantially identical nucleotide sequences specific for the second portion of the one or more first and second target nucleic acid sequences.

6. An assay according to claim 5, wherein the assay is performed in a first and a second reaction vessel, a portion of the sample is contacted with the first subset of free probes in the first reaction vessel and a portion of the sample is contacted with the second subset of free probes in the second reaction vessel.

7. An assay according to claim 1, further comprising using a thermostable ligase for ligating the probes.

8. An assay according to claim 1, wherein a substantially same amount of at least one fluorescent dye is incorporated into each bound probe in a subset, and one subset of spectrally-addressable bound probes is distinguishable from other subsets of spectrally-addressable bound probes based at least on the relative amount of the at least one fluorescent dye incorporated into the spectrally-addressable bound probe of the subset.

~~13.~~ An assay according to claim 1, wherein the assay further comprises contacting the sample with polymerase chain reaction components and amplifying the target nucleic acid molecule.

~~14.~~ A microsphere-based oligonucleotide ligation assay comprising:

(a) contacting a sample, which is suspected of containing target nucleic acid molecules having a certain nucleotide sequence, with a mixture comprising at least one set of free probes and at least one subset of microspheres to which are coupled bound probes, wherein (i) the free probes of a given set comprise a detectable label at one of their ends and an oligonucleotide having a predetermined nucleotide sequence that is complementary to at least a portion of the target nucleic acid molecules, (ii) the bound probes of a given subset of microspheres comprise a modifier moiety, which is used for coupling a bound probe to a microsphere, at one of their ends and an oligonucleotide having a predetermined nucleotide sequence that is complementary to at least another portion of the target nucleic acid molecules, and (iii) the microspheres of a given subset having a unique spectral address, which allows one to distinguish the microspheres of a given subset from those of another;

(b) allowing the free probes and the bound probes to hybridize to the target nucleic acid molecules,

(c) ligating one of the ends of the free probes with one of the ends of the bound probes to provide microsphere-bound ligated products; and

(d) detecting the presence of microsphere-bound ligated products.

11 15. The assay of claim 14 in which the free probes and the bound probes are allowed to hybridize to different portions of the target nucleic acid molecules.

12 16. The assay of claim 15 in which the different portions of the target nucleic acid molecules do not overlap.

13 17. The assay of claim 14 in which the free probes further comprise a phosphate at the other of their ends.

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18. The assay of claim 14 in which the bound probes further comprise a phosphate at the other of their ends.

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19. The assay of claim 14 in which the mixture comprises at least two subsets of microspheres, the bound probes coupled to the microspheres of one subset being different from those coupled to the microspheres of the other subset.

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20. The assay of claim 19 in which the bound probes differ in that the nucleotide found at one end of one subset differs from that found at the corresponding end of the other subset, wherein the nucleotide sequences comprising the at least two subsets of bound probes are otherwise substantially identical.

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21. The assay of claim 20 in which the mixture comprises free probes having substantially identical nucleotide sequences.

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22. The assay of claim 19 in which the bound probes differ in the identity of one or more nucleotides at one or more positions of the predetermined nucleotide sequence.

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23. The assay of claim 19 in which the bound probes differ due to one or more substitutions, insertions, deletions, or combinations thereof, at one or more positions of the predetermined nucleotide sequence.

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24. The assay of claim 15 in which the mixture comprises at least two sets of free probes, the nucleotide and the detectable label found at opposite ends of one set differing from the nucleotide and the detectable label found in the corresponding ends of the other set, wherein the nucleotide sequences comprising the at least two sets of free probes are otherwise substantially identical.

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25. The assay of claim 24 in which the mixture comprises bound probes having substantially identical nucleotide sequences.

26. The assay of claim 14 in which the oligonucleotides of the at least one set of free probes and at least one subset of microspheres have 5' and 3' ends, and wherein the free probes of a given set include a phosphate at their 5' ends and a detectable label at their 3' ends; and the modifier moiety is an amine which couples the 5' end of the oligonucleotide of the bound probe to a carboxylic acid group on the microsphere.

27. The assay of claim 26 in which the mixture comprises at least two subsets of microspheres, the bound probes coupled to the microspheres of one subset being different from the bound probes coupled to the microspheres of the other subset in that a portion of the oligonucleotide at the 3' end of one subset differs from the a portion of the oligonucleotide at the 3' end of the other subset, wherein the nucleotide sequences comprising the at least two subsets of bound probes are otherwise substantially identical.

28. The assay of claim 26 in which the mixture comprises at least two sets of free probes, the portion of the oligonucleotide found at the 5' end of one set differing from the portion of the oligonucleotide at the 5'ends of the other set, wherein the nucleotide sequences comprising the at least two sets of free probes are otherwise substantially identical.

29. The assay of claim 26 which is carried out in substantially the same reaction vessel.

30. The assay of claim 26 which is carried out in separate reaction vessels, at least one for each set of free probes.

31. The assay of claim 27 in which the microspheres of one subset can be distinguished from the microspheres of the other subset in that the microspheres of the one subset harbor at least one fluorescent dye at a concentration which differs from the concentration of the at least one fluorescent dye harbored by the microspheres of the other subset.

33. The assay of claim 27 in which the spectrally addressable microspheres of one subset can be distinguished from the spectrally addressable microspheres of another subset by the relative amounts of at least two fluorescent dyes harbored by the spectrally addressable microspheres.

34. The assay of claim 26, wherein the mixture further comprises polymerase chain reaction components, and wherein the assay further comprises the step of amplifying a portion of the target nucleic acid molecule.

35. A kit for performing a microsphere-based oligonucleotide ligation assay comprising bound probes coupled to spectrally addressable microspheres and free probes bearing a detectable label.

36. The kit of claim 35 in which at least two subsets of microspheres are present.

37. The kit of claim 36 which further comprises a thermostable ligase.

38. The kit of claim 36 which further comprises one or more reagents for effecting nucleic acid amplification.

39. The kit of claim 38 which further comprises one or more reaction buffers.

40. The assay of claim 14 in which the modifier moiety comprises an amine modifier moiety.

41. The assay of claim 14 in which the modifier moiety comprises a primary amine group for coupling the bound probe to a carboxylic acid group of the microsphere.